

CLAIMS

What is claimed is:

- 5
1. A library of yeast expression vectors encoding a library of fusion proteins, each vector comprising:
- 10
- a first nucleotide sequence encoding a first polypeptide subunit;
- a second nucleotide sequence encoding a second polypeptide subunit;
- and
- a linker sequence encoding a linker peptide that links the first
- nucleotide sequence and the second nucleotide sequence;
- wherein
- the first polypeptide subunit, the second polypeptide subunit,
- and the linker polypeptide are expressed as a single fusion protein within the
- library of fusion proteins; and
- 15
- the first and second nucleotide sequences each independently varies within the library of expression vectors.
2. The library of claim 1, wherein the yeast expression vector is a
- 20
- 2 μ plasmid vector.
3. The library of claim 1, wherein the yeast expression vector is a yeast-
- bacterial shuttle vector which contains a bacterial origin of replication.
4. The library of claim 1, wherein the diversity of the first or the second
- 25
- polypeptide subunit within the library of fusion proteins is at least 10³.
5. The library of claim 1, wherein the diversity of the first or the second
- polypeptide subunit within the library of fusion proteins is at least 10⁴.

6. The library of claim 1, wherein the diversity of the first or the second polypeptide subunit within the library of fusion proteins is at least 10^5 .

7. The library of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least 1×10^6 .

8. The library of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least 1×10^{10} .

9. The library of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least 1×10^{12} .

10. The library of claim 1, wherein the diversities of the first and second polypeptide subunits are each independently derived from libraries of precursor sequences that are not specifically designed for a target peptide or protein.

11. The library of claim 1, wherein the diversities of the first and second polypeptide subunits are not derived from one or more proteins that are known to bind to a target peptide or protein.

12. The library of claim 1, wherein the diversities of the first and second polypeptide subunits are not generated by mutagenizing one or more proteins that are known to bind to a target peptide or protein.

13. The library of claim 1, wherein the first and the second polypeptide subunits are subunits of a multimeric protein whose sequence varies within a library of multimeric proteins.

11
14. The library of claim 13, wherein the library of multimeric proteins are selected from the group consisting of libraries of antibodies, growth factor receptors, T cell receptors, cytokine receptors, tyrosine kinase-associated receptors, and MHC proteins.

5 12
15. The library of claim 1, wherein the first nucleotide sequence is 5' relative to the second nucleotide sequence.

13 12
16. The library of claim 15, wherein the first nucleotide sequence in the library of expression vectors comprises a coding sequence of an antibody heavy-chain variable region, and the second nucleotide sequence comprises a coding sequence of an antibody light-chain variable region.

14 13
17. The library of claim 16, wherein the source of the coding sequences of the antibody light-chain and heavy-chain variable regions is from human, non-human primate, or rodent DNA.

15 15 13
18. The library of claim 16, wherein the source of the coding sequences of the antibody light-chain and heavy-chain variable regions is from one or more non-immunized animals.

16 13
19. The library of claim 16, wherein the source of the coding sequences of the antibody light-chain and heavy-chain variable regions are selected from the group consisting of human fetal spleen, lymph nodes or peripheral blood cells.

20. The library of claim 1, wherein the linker peptides expressed by the library of expression vectors provide a substantially conserved conformation between the first and second polypeptide subunits across the library of fusion

proteins expressed by the library of expression vectors.

21. The library of claim 1, wherein the conformation of the fusion protein having the first and second polypeptide subunits linked by the linker peptide mimics a conformation of a single chain antibody.

- ~~17~~
22. The library of claim 1, wherein the linker sequences in the library of expression vectors are between 30-120 bp in length.

- ~~14~~
10 23. The library of claim 1, wherein the linker sequences in the library of expression vectors are between 45-102 bp in length.

- ~~19~~
24. The library of claim 1, wherein the linker sequence in the library of expression vectors are between 45-63 bp in length.

- 15
~~20~~
25. The library of claim 1, wherein the linker sequences in the library of expression vectors comprise a nucleotide sequence encoding an amino acid sequence of Gly-Gly-Gly-Gly-Ser in 3 or 4 tandem repeats.

- ~~21~~
20 26. The library of claim 1, wherein each of the expression vectors further comprises a sequence encoding an affinity tag.

- ~~22~~
27. The library of claim 26, wherein the affinity tag is selected from the group consisting of a polyhistidine tag, polyarginine tag, glutathione-S-transferase, maltose binding protein, staphylococcal protein A tag, and an EE-epitope tag.

28. A library of expression vectors, each vector comprising:
a transcription sequence encoding an activation domain or a DNA

binding domain of a transcription activator;

a first nucleotide sequence encoding a first polypeptide subunit;

a second nucleotide sequence encoding a second polypeptide subunit;

and

5 a linker sequence encoding a linker peptide that links the first
nucleotide sequence and the second nucleotide sequence;

wherein

the activation domain or the DNA binding domain of the
transcription activator, the first polypeptide subunit, the second polypeptide
10 subunit, and the linker polypeptide are expressed as a single fusion protein;
and

the first and second nucleotide sequences each independently
varies within the library of expression vectors.

15 29. The library of claim 28, wherein the expression vector is selected from
the group consisting of bacterial, phage, yeast, mammalian and viral
expression vectors.

20 30. The library of claim 28, wherein the expression vector is a 2 μ plasmid
yeast expression vector.

31. The library of claim 28, wherein the transcription sequence is 5' relative
to the first nucleotide sequence, the linker sequence, and the second
nucleotide sequence.

25 32. The library of claim 28, wherein the transcription sequence is 3' relative
to the first nucleotide sequence, the linker sequence, and the second
nucleotide sequence.

remains.

ΦΙ

2007
Jul 23 10 35 a lib

24
~~36.~~

25
37.

25 ~~26~~
38.